

In re Application of:  
Hiroaki Shizuya  
Application No.: 10/659,034  
Filed: September 9, 2003  
Page 2

PATENT  
Attorney Docket No.: CIT1620-1

**Amendments to the Claims**

Please amend claims 1, 8, 11, 14, 16-18, 23-25, 32, 35, and 38-41 as indicated in the listing of claims.

Please cancel claims 2, 15, 26, and 42 without prejudice or disclaimer.

Please add new claims 43-46.

The listing of claims will replace all prior versions, and listings of claims in the application:

**Listing of Claims:**

Claim 1. (Currently Amended) A method of generating a humanized ~~animal~~mouse, comprising:

recombining a first DNA construct with a second DNA construct,  
wherein the first DNA construct has a ~~non-human-animal~~murine DNA sequence contained therein, and  
wherein the second DNA construct has a human DNA sequence contained therein and the human DNA sequence is flanked by a first and a second ~~non-human-animal~~murine DNA sequence;  
isolating a recombined third DNA construct having a the human DNA sequence flanked by the first and second ~~non-human-animal~~murine DNA sequences; and  
introducing the recombined third DNA construct into a ~~non-human~~murine embryogenic stem cell;

In re Application of:  
Hiroaki Shizuya  
Application No.: 10/659,034  
Filed: September 9, 2003  
Page 3

PATENT  
Attorney Docket No.: CIT1620-1

introducing the embryogenic stem cells into a mouse blastocyst, thereby producing a chimeric blastocyst;  
implanting the chimeric blastocyst into a pseudopregnant mouse, wherein the pseudopregnant mouse delivers a humanized mouse, thereby generating a humanized mouse.

Claim 2. (Cancel)

Claim 3. (Original) The method of claim 1, wherein the first DNA construct is a bacterial artificial chromosome.

Claim 4. (Original) he method of claim 1, wherein the second DNA construct is a bacterial artificial chromosome.

Claim 5. (Original) The method of claim 4, wherein the bacterial artificial chromosome is linearized.

Claim 6. (Original) The method of claim 1, wherein the recombining is carried out in a strain of *E. coli*.

Claim 7. (Original) The method of claim 1, wherein the *E. coli* is deficient for *sbcB*, *sbcC*, *recB*, *recC* or *recD* activity and has a temperature sensitive mutation in *recA*.

Claim 8. (Currently Amended) The method of claim 1, wherein the human ~~gene~~DNA sequence is selected from the group consisting of ~~genes encoding~~a G-protein coupled receptor[[s]] gene, a kinase[[s]] gene, a phosphatase[[s]] gene, an ion channel[[s]] gene, a nuclear receptor[[s]] gene, an oncogene[[s]] gene, a cancer suppressor gene[[s]] gene, a viral

In re Application of:  
Hiroaki Shizuya  
Application No.: 10/659,034  
Filed: September 9, 2003  
Page 4

PATENT  
Attorney Docket No.: CIT1620-1

receptor[[s]]\_gene, a bacterial receptor[[s]]\_gene, a P450 gene[[s]]\_gene, an insulin receptor[[s]]  
gene, an immunoglobulin[[s]]\_gene, a metabolic pathway gene[[s]], a transcription factor[[s]]  
gene, a hormone receptor[[s]]\_gene, a cytokine[[s]]\_gene, a cell signaling pathway gene[[s]] and a  
cell cycle gene[[s]].

Claim 9. (Original) The method of claim 1, wherein the third DNA construct is a bacterial  
artificial chromosome.

Claim 10. (Original) The method of claim 1, wherein the human DNA sequence is a human  
gene sequence having at least one intron contained therein.

Claim 11. (Currently Amended) The method of claim 1, wherein the third DNA construct  
has a selection marker contained within the at least one intron.

Claim 12. (Original) The method of claim 11, wherein the selection marker is added  
following the recombining step.

Claim 13. (Original) The method of claim 11, wherein the selection marker is a positive  
selection marker.

Claim 14. (Currently Amended) The method of claim 11, wherein the third DNA construct  
has a second selection marker that flanks the first or the second ~~non-human animal~~ murine-DNA  
sequence.

Claim 15. (Cancel)

In re Application of:  
Hiroaki Shizuya  
Application No.: 10/659,034  
Filed: September 9, 2003  
Page 5

PATENT  
Attorney Docket No.: CIT1620-1

Claim 16. (Currently Amended) The method of claim 1, wherein the human DNA coding sequence comprises a start codon having a 5' end, the human DNA sequence and the first non-human mouse DNA sequence in the second construct are is joined to the human DNA coding sequence at the 5' end of a the start codon in a human gene coding sequence.

Claim 17. (Currently Amended) The method of claim 16, wherein the human DNA coding sequence comprises a stop codon having a 3' end, the human DNA sequence and the second non-human mouse DNA sequence in the second construct are is joined to the human DNA coding sequence at the 3' end of a the stop codon in the human gene coding sequence.

Claim 18. (Currently Amended) A DNA construct for performing homologous recombination within a cell, the construct comprising:

- a human DNA coding sequence having at least one intron disposed therein;
- a selection marker gene contained within said at least one intron;
- a first non-human animal DNA sequence and a second non-human animal DNA sequence, wherein said first and second non-human animal DNA sequences flanking the human DNA coding sequence, wherein the first and second non-human animal flanking DNA sequences are homologous to sequences in the genome of the non-human animal that flank a gene orthologous to the human DNA coding sequence.

Claim 19. (Original) The DNA construct of claim 18, further comprising a second selection marker adjacent to one of the non-human DNA sequences.

In re Application of:  
Hiroaki Shizuya  
Application No.: 10/659,034  
Filed: September 9, 2003  
Page 6

PATENT  
Attorney Docket No.: CIT1620-1

Claim 20. (Original) The DNA construct of claim 18, wherein the construct is a linearized bacterial artificial chromosome.

Claim 21. (Original) The DNA construct of claim 18, wherein the first and second non-human DNA sequences are mouse genomic DNA sequences.

Claim 22. (Original) The DNA construct of claim 18, wherein the flanking sequences are from about 0.1 to 200 kb in length.

Claim 23. (Currently Amended) The DNA construct of claim 18, wherein the human DNA coding sequence comprises a start codon having a 5' end ~~human DNA coding sequences and the first non-human sequence are~~ is joined adjacent to the 5' end of the start codon of the human DNA coding sequence.

Claim 24. (Currently Amended) The DNA construct of claim 18, wherein the human DNA coding sequence comprises a stop codon having a 3' end ~~human DNA coding sequences and the first non-human sequence are~~ is joined adjacent to the 3' end of the stop codon of the human DNA coding sequence.

Claim 25. (Currently Amended) A method for generating a DNA construct for performing homologous recombination within a cell by

recombining a first DNA construct with a second DNA construct,  
wherein the first DNA construct has a non-human animal DNA sequence contained therein,

In re Application of:  
Hiroaki Shizuya  
Application No.: 10/659,034  
Filed: September 9, 2003  
Page 7

PATENT  
Attorney Docket No.: CIT1620-1

wherein the second DNA construct has a human DNA sequence contained therein and the human DNA sequence is flanked by a first and a second non-human animal DNA sequence; and  
isolating a recombined third DNA construct having ~~a~~ the human DNA sequence flanked by the first and second non-human animal DNA sequence; ~~and~~  
~~introducing the recombined third DNA construct into a non-human embryogenic stem cell.~~

Claim 26. (Cancel)

Claim 27. (Original) The method of claim 25, wherein the first DNA construct is a bacterial artificial chromosome.

Claim 28. (Original) The method of claim 25, wherein the second DNA construct is a bacterial artificial chromosome.

Claim 29. (Original) The method of claim 28, wherein the bacterial artificial chromosome is linearized.

Claim 30. (Original) The method of claim 25, wherein the recombining is carried out in a strain of *E. coli*.

Claim 31. (Original) The method of claim 25, wherein the *E. coli* is deficient for *sbcB*, *sbcC*, *recB*, *recC* or *recD* activity and has a temperature sensitive mutation in *recA*.

Claim 32. (Currently Amended) The method of claim 25, wherein the human ~~gene~~ DNA sequence is selected from the group consisting of ~~genes encoding a~~ G-protein coupled

In re Application of:  
Hiroaki Shizuya  
Application No.: 10/659,034  
Filed: September 9, 2003  
Page 8

PATENT  
Attorney Docket No.: CIT1620-1

receptor[[s]] gene, a kinase[[s]] gene, a phosphatase[[s]] gene, an ion channel[[s]] gene, a nuclear receptor[[s]] gene, an oncogene[[s]] gene, a cancer suppressor gene[[s]] gene, a viral receptor[[s]] gene, a bacterial receptor[[s]] gene, a P450 gene[[s]] gene, an insulin receptor[[s]] gene, an immunoglobulin[[s]] gene, a metabolic pathway gene[[s]], a transcription factor[[s]] gene, a hormone receptor[[s]] gene, a cytokine[[s]] gene, a cell signaling pathway gene[[s]] and a cell cycle gene[[s]].

Claim 33. (Original) The method of claim 25, wherein the third DNA construct is a bacterial artificial chromosome.

Claim 34. (Original) The method of claim 25, wherein the human DNA sequence is a human gene sequence having at least one intron contained therein.

Claim 35. (Currently Amended) The method of claim 25, wherein the third DNA construct has a selection marker contained within the at least one intron.

Claim 36. (Original) The method of claim 35, wherein the selection marker is added following the recombining step.

Claim 37. (Original) The method of claim 35, wherein the selection marker is a positive selection marker.

Claim 38. (Currently Amended) The method of claim 35, wherein the third DNA construct has a second selection marker that flanks the first or the second non-human animal DNA sequence.

In re Application of:  
Hiroaki Shizuya  
Application No.: 10/659,034  
Filed: September 9, 2003  
Page 9

PATENT  
Attorney Docket No.: CIT1620-1

Claim 39. (Currently Amended) The method of claim 25, wherein the human DNA sequence comprises a start codon having a 5' end, and the first non-human DNA sequence in the second construct ~~are~~is joined to the human DNA coding sequence at the 5' end of a~~the~~ start codon ~~in a human gene coding sequence~~.

Claim 40. (Currently Amended) The method of claim 39, wherein the human DNA sequence comprises a stop codon having a 3' end, and the second non-human DNA sequence in the second construct ~~are~~is joined to the 3' of ~~a~~the stop codon ~~in the human gene coding sequence~~.

Claim 41. (Currently Amended) A humanized ~~animal~~mouse produced by the method of claim 1, wherein the human DNA sequence is selected from a G-protein coupled receptor gene, a kinase gene, a phosphatase gene, an ion channel gene, a nuclear receptor gene, an oncogene gene, a cancer suppressor gene, a viral receptor gene, a bacterial receptor gene, a P450 gene, an insulin receptor gene, an immunoglobulin gene, a metabolic pathway gene, a transcription factor gene, a hormone receptor gene, a cytokine gene, a cell signaling pathway gene and a cell cycle gene.

Claim 42. (Cancel)

Claim 43. (New) The humanized mouse of claim 41, wherein the gene is involved in human drug metabolism.



In re Application of:  
Hiroaki Shizuya  
Application No.: 10/659,034  
Filed: September 9, 2003  
Page 10

PATENT  
Attorney Docket No.: CIT1620-1

Claim 44. (New) The humanized mouse of claim 41, wherein the gene is a PXR, CAR, RXR, CYP3A4, CYP2B6, CYP2C9 or MDR1 gene.

Claim 45. (New) A method of generating a humanized cell, comprising:

recombining a first DNA construct with a second DNA construct, wherein the first DNA construct has a non-human animal DNA sequence contained therein, and wherein the second DNA construct has a human DNA sequence contained therein and the human DNA sequence is flanked by a first and a second non-human animal DNA sequence; isolating a recombined third DNA construct having a the human DNA sequence flanked by the first and second non-human animal DNA sequence; and

introducing the recombined third DNA construct into a non-human animal cell, thereby generating a humanized cell.

Claim 46. (New) The method of claim 45, wherein the non-human animal cell is an embryogenic stem cell.